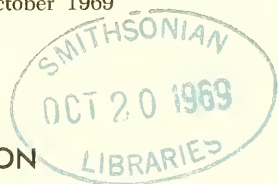


QH
1
B4X
NH

2

3 October 1969

PROCEEDINGS
OF THE
BIOLOGICAL SOCIETY OF WASHINGTON



USE OF MALE EXTERNAL GENITALIC DETAILS AS
TAXONOMIC CHARACTERS IN SOME SPECIES OF
PALAEEMONETES (DECAPODA, PALAEMONIDAE)¹

BY LAURENCE E. FLEMING
*Mississippi State University*²

The various species of shrimp belonging to the genus *Palaemonetes* are currently identified and classified on the basis of the spinose ornamentation of several different body sites. Because of overlap and variation, identification and relationships are difficult to determine.

The taxonomic characters currently in use for *Palaemonetes*, with the possible exception of cheliped proportions, are probably subject to ecological modifications. This is especially true of spinose ornamentation. On the other hand, the structures utilized in amplexus in most Crustacea are usually stable and in so far as is known, are little affected by ecological factors. This is especially true in many Malacostraca where male appendages have been particularly useful in taxonomic and evolutionary studies.

In this study the external genital details of the second pleopod of male *Palaemonetes* were examined and evaluated as taxonomic characters for identification. The study was limited to the epigeal species of the Gulf Coastal Plain of the United States and its coastal waters. Stable, and unique for the species, setal ornamentation of the tip of the appendix masculina of the second pleopod and equally stable gross morphology of the second pleopod provide potentially valuable characteristics for the systematic study of Palaemonidae.

¹ This research was supported in part by NSF Grant No. GB-4719 to J. F. Fitzpatrick, Jr.

² Present address: Department of Biology, Virginia Polytechnic Institute, Blacksburg, Virginia.

Species Studied: Holthuis (1949, 1952) listed six epigeal species of *Palaemonetes* as occurring in the United States, and five of these are known from the state of Mississippi and adjacent waters (*ibid.*): *P. (P.) kadiakensis* Rathbun, 1902; *P. (P.) paludosus* (Gibbes, 1850); *P. (P.) vulgaris* (Say, 1818); *P. (P.) pugio* Holthuis, 1949; *P. (P.) intermedius* Holthuis, 1949. Specimens of these species from Mississippi were the principal source of information although most specimens of *P. paludosus* were collected in St. Charles and St. James parishes, Louisiana.

Additional material was available from various sources, and specimens from four Gulf states (Mississippi, Louisiana, Texas, and Alabama) were analyzed. Material studied included five collections from two counties in Alabama, 21 collections from five parishes in Louisiana, two collections from two counties in Texas and 42 collections from 15 counties in Mississippi.

Following initial evaluations, type specimens of these species at the Smithsonian Institution were examined and the conclusions validated. Other types at SI were also examined for the same characteristics to determine if the proposed criteria could be applied to other species of the genus. These species, studied by means of type material at SI, are: *P. (P.) schmitti* Holthuis; *P. (P.) suttkusi* Smalley; *P. (Alaocaris) antrorum* Benedict. Type material of *P. (P.) eigenmanni* Hay and *P. (P.) hiltoni* Schmitt were also examined at SI, but the pleopods were disarticulated, shriveled or in some way damaged and little use could therefore be made of this material.

Procedures: Specimens of *Palaemonetes* were collected by means of dip nets, seines, and on occasion, use was made of boat-drawn trawls and plankton nets. The specimens were collected from large and small ponds, roadside sloughs, running streams, inlet bayous of the Mississippi Sound of the Gulf of Mexico and from the Gulf of Mexico itself. Freshwater species were found to be the most abundant in floating, submerged and emergent aquatic vegetation such as duckweed (*Spirodela* sp. and *Lemna* sp.), water hyacinth (*Eichhornia* sp.), pondweed (*Potamogeton* spp.) and bladderwort (*Utricularia* sp.). Brackish and marine species were mostly obtained from growths of eelgrass (*Vallisneria* spp.).

A total of 1582 specimens were studied in detail: 845 specimens of *P. kadiakensis*, 169 specimens of *P. paludosus*, 430 specimens of *P. pugio*, 71 specimens of *P. vulgaris*, and 67 specimens of *P. intermedius*.

Individuals were randomly selected and the second left pleopods removed and temporarily mounted on slides using Monk's Mounting Medium (5cc corn syrup, 5cc fruit pectin, 3cc of water with thymol added as a preservative). Each pleopod was placed in precisely the same position on the slide. Then a careful examination of each pleopod was made using a compound microscope. To further validate the conclusions drawn, several pleopods were dismounted from their position on the slide, rearranged in exactly the opposite position, and again subjected to microscopic examination.

Concluding these studies, camera lucida drawings were made of selected

representative pleopods. Two sets of drawings were made of each. One drawing was made under the low power ($35\times$) of a compound microscope to reveal the structure of the entire pleopod, and another drawing was made under the high power ($450\times$) of the tip of the appendix masculina of each male specimen. The coverslips of the slides were then ringed with "Permout" to insure their permanency.

Following this, *in situ* camera lucida drawings were made of the second right pleopod of randomly selected specimens using a magnification of $38\times$ on a stereoscopic microscope. This was done to validate the accuracy of the orientations and interpretations of disarticulated appendages.

OBSERVATIONS

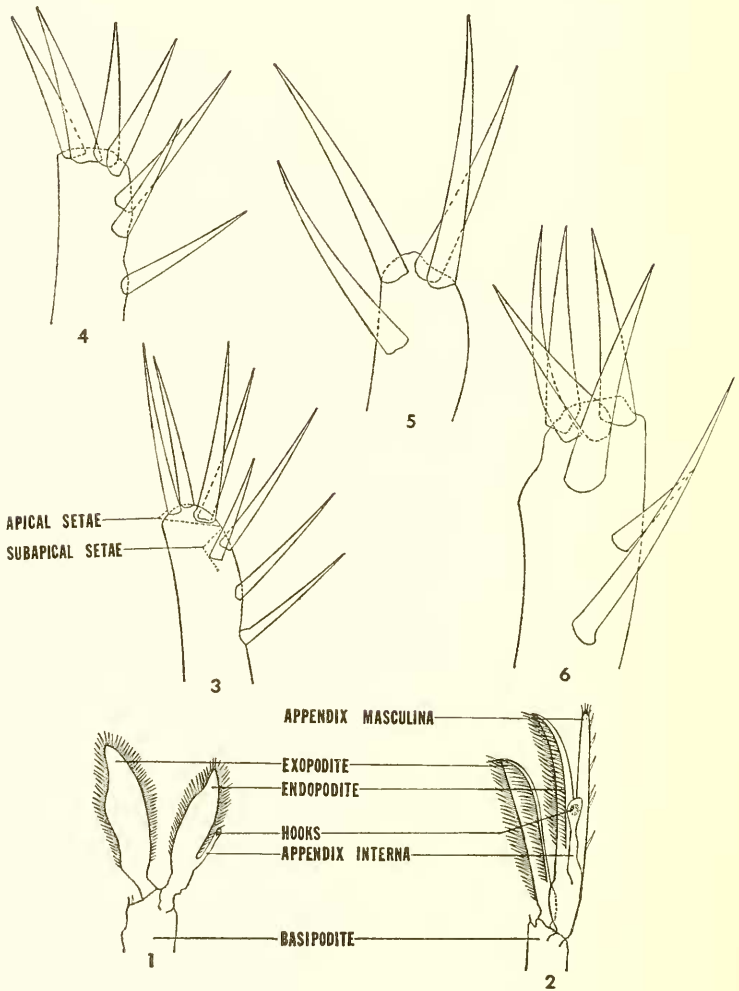
In all of the specimens examined, the general appearance of the external secondary sex characters of the five different species seem to resemble Meehan's (1936) description of those of *P. paludosus* (probably *P. kadiakensis*). In *Palaemonetes* the first two pairs of pleopods exhibit sexual dimorphism and modification. The first pair of pleopods (which are not copulatory organs) are relatively unmodified but the inner ramus (= endopodite proper) of the male is approximately three times as long as that of the female. In both sexes the endopodite and exopodite are heavily armed with plumose setae. The second pair of pleopods (the pair exhibiting the greater modification) have the endopodite modified to include a non-podomere appendix interna (= retinaculum of Meehan, 1936) arising from the inner margin in both sexes (Figs. 1 and 2). Between the endopodite proper and the appendix interna of the male an accessory process arises near the base of the appendix interna and is termed the appendix masculina (*op. cit.*).

Interspecific variability of the second pleopod of male specimens of *Palaemonetes* were found. The gross morphology of the various parts of the pleopod varies in such factors as proportional lengths of certain parts, shape of the tip of the appendix interna and overall shape of the appendix masculina. Another species specific feature is the setal ornamentation of the tip of the appendix masculina. The number of apical setae and the number of subapical setae are sufficient to identify most species, except *P. vulgaris* and *P. intermedius* which are identical in this respect.

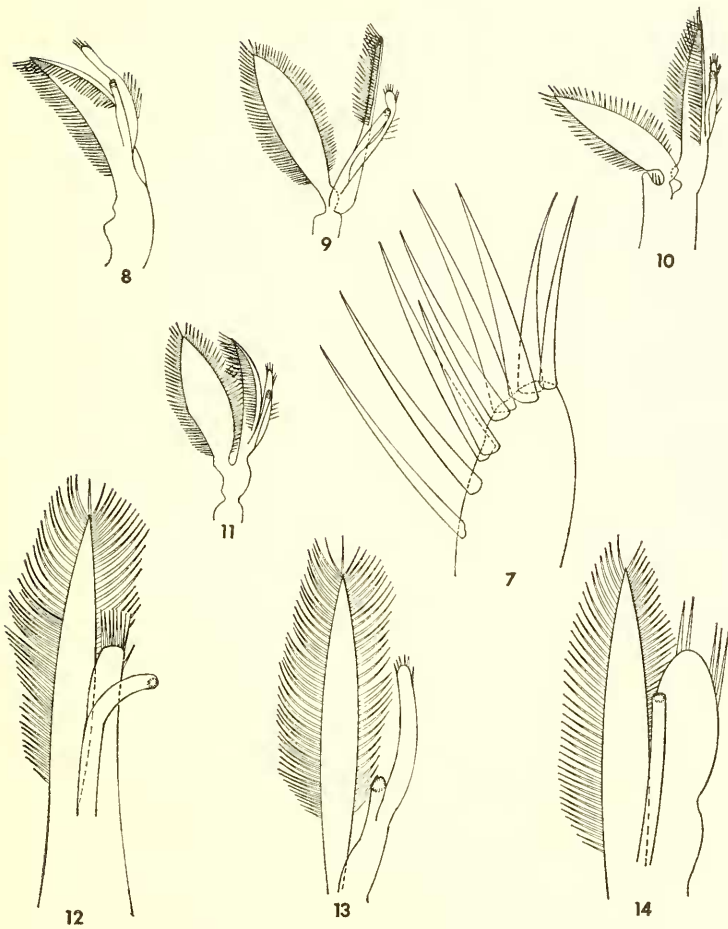
P. intermedius (Fig. 3) is used as an example to illustrate the position of the apical setae in reference to the subapical setae. There are four apical setae and two subapical setae in *P. intermedius* and *P. vulgaris* (Fig. 4). There are three apical setae and one subapical seta in *P. kadiakensis* (Fig. 5); four apical setae and one subapical seta in *P. paludosus* (Fig. 6); five apical and one or two subapical setae in *P. pugio* (Fig. 7).

In summary, the setal ornamentation of the appendix masculina is specifically unique in the species of *Palaemonetes* examined, with the one exception, as stated above, involving *P. vulgaris* and *P. intermedius*. All the other species are easily separable using this character alone.

Study of the gross morphology of the pleopod reveals differences in proportions of two structures: (1) extent of the appendix interna along the



FIGS. 1-6. Second pleopod of various species of *Palaemonetes*. 1. Mesial view of *P. kadiakensis* Rathbun illustrating gross morphology of a female pleopod. 2. Mesial view of *P. paludosus* (Gibbes) illustrating gross morphology of a male pleopod. 3. Tip of appendix masculina of *P. intermedius* Holthuis showing setal ornamentation. 4. Tip of appendix masculina of *P. vulgaris* (Say) showing setal ornamentation. 5. Tip of appendix masculina of *P. kadiakensis* Rathbun showing setal ornamentation. 6. Tip of appendix masculina of *P. paludosus* (Gibbes) showing setal ornamentation.



FIGS. 7-14. Second male pleopod of various species of *Palaemonetes*. 7. Tip of appendix masculina of *P. pugio* Holthuis showing setal ornamentation. 8. Mesial view of *P. kadiakensis* Rathbun illustrating specific gross morphology; exopodite concealed. 9. Mesial view of *P. pugio* Holthuis illustrating specific gross morphology. 10. Mesial view of *P. vulgaris* (Say) illustrating specific gross morphology. 11. Mesial view of *P. intermedius* Holthuis illustrating specific gross morphology. 12. Mesial view of *P. schmitti* Holthuis (from the Canal Zone) illustrating specific gross morphology. 13. Mesial view of *P. suttkusi* Smalley (from Mexico) illustrating specific gross morphology. 14. Mesial view of *P. antrorum* Benedict (a subterranean species) illustrating specific gross morphology.

appendix masculina and (2) extent of the appendix masculina along the endopodite proper.

The appendix interna extends along slightly more than the proximal one-half of the appendix masculina in *P. kadiakensis* (Fig. 8); along less than the proximal one-half of the appendix masculina in *P. paludosus* (Fig. 2); along the proximal three-fourths of the appendix masculina, and in some specimens the appendices may even be subequal in *P. vulgaris* (Fig. 10). In *P. pugio* (Fig. 9) and *P. intermedius* (Fig. 11) the appendix interna extends along the proximal two-thirds of the appendix masculina. Through the use of this character all species except *P. pugio* and *P. intermedius* can be distinguished.

The appendix masculina extends to the distal one-third of the endopodite proper in *P. vulgaris* (Fig. 10) and *P. pugio* (Fig. 9); to the distal one-fourth of the endopodite proper in *P. intermedius* (Fig. 11); to the distal one-eighth of the endopodite proper in *P. kadiakensis* (Fig. 8); to the distal one-tenth of the endopodite proper, and in some specimens the two appendices are subequal in *P. paludosus* (Fig. 2). Thus, *P. vulgaris* and *P. pugio* are alike in this character.

The shape of the tip of the appendix interna in *P. paludosus* (Fig. 2) is large and flat or paddle-shaped while in all other species it is small and round.

The overall shape of the appendix masculina differs in two species from the shape of the structure in the other three species. In *P. kadiakensis* (Fig. 8) and in *P. pugio* (Fig. 9) the appendix masculina is gently curved laterad, with the curve in both species occurring in the approximate area of the limit of extent of the appendix interna along the appendix masculina. In *P. paludosus* (Fig. 2), *P. vulgaris* (Fig. 10), and *P. intermedius* (Fig. 11) the appendix masculina is straight. In *P. paludosus* the appendix masculina is straight and stiffened in comparison with the appendix masculina of *P. vulgaris* and *P. intermedius*.

A summary of the various features of the second pleopod of these five species is given in Table 1. The types of all are comparable with the figures (Figs. 1-11) and Table 1.

Type material from areas outside of the Gulf Coastal Plain was examined at SI and found to exhibit species unique characteristics. In *P. schmitti* (Fig. 12) there is a very complicated setal ornamentation (eight long apical setae); one short subapical seta; and the appendix interna turns upward and outward. In *P. suttkusi* (Fig. 13) there are five very short apical setae arranged in a circle; one short subapical seta; and the appendix interna is very short and straight. In *P. antrorum*, a subterranean species, (Fig. 14) there are two long apical setae; three long subapical setae; the appendix interna is relatively straight; and the appendix masculina is very stout.

DISCUSSION

Although Holthuis' revision (1952) of the subfamily Palaemoninae now serves as the primary source for all taxonomic work in this group, specific,

TABLE 1. Characteristics of second pleopod of certain species of *Palaemonetes*.

Species	Appendix masculina			Appendix interna		
	Num-ber of apical setae	Num-ber of sub-apical setae	Shape of tip	Extent along endopodite proper	Shape of tip	Extent along appendix masculina
<i>kadiakensis</i>	3	1	curved	To proximal $\frac{7}{8}$	small, round	slightly more than proximal $\frac{1}{2}$
<i>paludosus</i>	4	1	straight	To proximal $\frac{9}{10}$	large, flat or paddle-shaped	less than proximal $\frac{1}{2}$
<i>pugio</i>	5	1-2	curved	To proximal $\frac{2}{3}$	small, round	equal to proximal $\frac{2}{3}$
<i>vulgaris</i>	4	2	straight	To proximal $\frac{2}{3}$	small, round	slightly more than proximal $\frac{3}{4}$
<i>intermedius</i>	4	2	straight	To proximal $\frac{3}{4}$	small, round	equal to proximal $\frac{2}{3}$
<i>schmitti</i>	8	1	straight	To proximal $\frac{2}{3}$	small, round	slightly more than proximal $\frac{3}{4}$
<i>suttkusi</i>	5	1	curved	To proximal $\frac{5}{6}$	small, round	less than proximal $\frac{1}{2}$
<i>antrorum</i>	2	3	straight	To proximal $\frac{2}{3}$	small, round	equal to proximal $\frac{2}{3}$

subgeneric and generic characters utilized by him exhibit overlap, variability and, in some cases, indistinctiveness which tend to make the identification of certain specimens extremely difficult. Some characters are difficult to evaluate. For example, the pleura of the fifth abdominal segments are used as a diagnostic character in the separation of *P. pugio*, *P. vulgaris*, and *P. intermedius* yet these features are intraspecifically mutable.

The use of the second pleopod of the male as a source of taxonomic characters in *Palaemonetes* would alleviate many difficulties now encountered in identifying species of *Palaemonetes* and in so doing would make a contribution to the taxonomy of the group. Further, evolutionary and interspecific relationships probably can be better evaluated with more stable characters.

Holthuis (1952) noted that *P. vulgaris*, *P. pugio* and *P. intermedius* are very similar and for a long time have been confused under the name *P. carolinus* and *P. vulgaris*. He listed the characters that he used to separate

them and then concluded by stating that, although the three species can be generally easily separated, there are examples, especially with juveniles, when identification of a specimen is difficult. I should note that the use of details of the male external genitalia in identification does not remove the difficulties in identifying juveniles and females; its value lies in the increased confidence its use imparts to identifications of adult male specimens.

The separation of *P. vulgaris* from *P. intermedius* is extremely difficult with the use of the characteristics of the appendix masculina. Differentiation between the two, in fact, can be attained solely through the use of the proportional lengths of the appendix interna and appendix masculina. Further, the significance of these two characters has yet to be determined, especially with reference to other characteristics of the two populations.

There is similarity in larval development of *P. pugio*, *P. intermedius*, and *P. vulgaris* (Broad, 1957; Broad and Hubschman, 1962). Broad and Hubschman (1963) working with *P. kadiakensis* larvae and Dobkins (1963) working with *P. paludosus* larvae found that the larval development of the freshwater forms could be distinguished from the salt water forms by the tendency toward condensation of the stages, larger eggs and larger larvae of the freshwater forms.

From this information, together with that gathered from studies on the male second pleopod, one concludes that *P. pugio*, *P. vulgaris*, and *P. intermedius* are indeed related. *P. pugio*, however, is conspicuously different and easily separable, and thus specifically distinct from either *P. vulgaris* or *P. intermedius*. *P. vulgaris* and *P. intermedius*, on the other hand, are visibly identical in such a varying array of features and perceptibly different in so few characters that they could be conspecific. This view cannot be supported satisfactorily or refuted until more information is provided, particularly detailed populational and ecological data. For the time being, at least, there are distinct morphological differences between the two and there are no evidences of interbreeding. Therefore, they are best considered specifically distinct. *P. kadiakensis* and *P. paludosus* are clearly morphologically distinct from each other and from the other species. Thus, they, too, represent separate species. Examination of type material at the United States National Museum confirmed the distinctiveness of the morphological features of the genital apparatus of the male second pleopod of the five species of *Palaemonetes* studied.

Epigeic species of *Palaemonetes* from areas outside the Gulf Coastal Plain of the United States apparently also are specifically unique in these characteristics as revealed by type specimens from Mexico and the Canal Zone examined at SI. Subterranean species of *Palaemonetes* probably also may be similarly identified if studies made on type material of *Palaemonetes* (*Alaocaris*) *antrorum* Benedict, 1896, at SI are any indication.

SUMMARY

1. The second pleopod of the male may be used in taxonomic evaluation of epigeic species of *Palaemonetes*.

2. *P. kadiakensis*, *P. paludosus* and *P. pugio* can be easily separated from one another, and all can be differentiated from *P. vulgaris* and *P. intermedius* using counts of apical and subapical setae of the tip of the appendix masculina; *P. vulgaris* and *P. intermedius* cannot be distinguished using this character.
3. The relative length of the appendix interna can be used to distinguish *P. kadiakensis*, *P. paludosus* and *P. vulgaris*, although *P. pugio* and *P. intermedius* are indistinguishable by this character.
4. The relative length of the appendix masculina is distinctive in *P. paludosus*, *P. kadiakensis* and *P. intermedius*, but similar in *P. vulgaris* and *P. pugio*.
5. The tip of the appendix interna is large and flat or paddle-shaped in *P. paludosus*; it is small and round in all other species.
6. The appendix masculina is curved laterad in *P. kadiakensis* and *P. pugio* and straight in all other species; it is decidedly stiffened in *P. paludosus*.
7. Studies of the second pleopod and statements of other students suggest that *P. intermedius* and *P. vulgaris* may be conspecific, but they are considered here as distinct species; all other species are clearly distinct.
8. Studies made on type specimens of *P. schmitti*, *P. suttkusi* and *P. antrorum* at USNM revealed that they likewise may be separated from each other and from other species by these criteria.

ACKNOWLEDGMENTS

The writer wishes to express his sincere appreciation to Dr. J. F. Fitzpatrick, Jr., for his assistance and supervision of this study and for reviewing the manuscript. Dr. Fitzpatrick was not only an invaluable counsel throughout the course of this research but a constant source of encouragement. Dr. Perry C. Holt, Virginia Polytechnic Institute, carefully read the manuscript and made helpful suggestions. Drs. Gordon Gunter and Walter Abbott generously allowed use of facilities at the Gulf Coast Research Laboratory, and Mr. J. Y. Christmas and Mr. G. P. Garwood made museum specimens available; Mrs. Shirley Dimmick provided numerous aids in securing literature and information on *Palaemonetes*. Dr. James Franklin Payne and Mr. Shih-ming Chien were generous with help in collecting specimens. Dr. M. Saeed Mulkana is especially thanked for his help and assistances far too numerous to enumerate. Dr. Roger F. Cressey, Division of Crustacea, Museum of Natural History, Smithsonian Institution, kindly made it possible for me to study type specimens of *P. schmitti*, *P. suttkusi* and *P. antrorum*.

LITERATURE CITED

- BROAD, A. C. 1957. Larval development of *Palaemonetes pugio* Holthuis. Biol. Bull. 112: 144-161.
- AND J. H. HUBSCHMAN. 1962. A comparison of larvae and larval development of species of eastern United States *Palae-*

- monetes* with special reference to the development of *Palaemonetes intermedius* Holthuis. Amer. Zool. 2: 394-395 (Abstract).
- . 1963. The larval development of *Palaemonetes kadiakensis* M. J. Rathbun in the laboratory. Amer. Microscop. Soc., Trans. 82: 185-197.
- DOBKIN, S. 1963. The larval development of *Palaemonetes paludosus* (Gibbes, 1850) (Decapoda, Palaemonidae), reared in the laboratory. Crustaceana, 6: 41-61.
- GIBBES, L. R. 1850. On the carcinological collections of the United States. Proc. Amer. Ass. Advance. Sci. 3: 167-201.
- HOLTHUIS, L. B. 1949. Note on the species of *Palaemonetes* (Crustacea, Decapoda) found in the United States of America. Proc. Konink. Nederland Akad. Wetensch. 52: 87-95.
- . 1952. A general review of the Palaemonidae (Crustacea, Decapoda, Natantia) of the Americas. II. The subfamily Palaemoninae. Occ. Pap. Allan Hancock Found. 12: 1-396.
- MEEHEAN, O. L. 1936. Notes on the freshwater shrimp *Palaemonetes paludosa* (Gibbes). Amer. Microscop. Soc., Trans. 55: 433-441.
- RATHBUN, M. J. 1902. Descriptions of new decapod crustaceans from the West Coast of North America. Proc. U.S. Nat. Mus. 24: 885-905.
- SAY, T. 1818. An account of the Crustacea of the United States. J. Acad. Nat. Sci., Philadelphia 2: 235-458.